Rapid probing for intrinsic traits related to cold stress tolerance at physiological, biophysical and biochemical levels in *Hevea brasiliensis*

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Abstract

Consequences of low temperature stress in two clones (RRIM 600 and RRII 105) of *Hevea brasiliensis* were comprehensively studied through critical assessment of metabolic responses at low temperature in comparison with the control plants grown in warm climate. As a reflection of the primary physiological state net CO_2 assimilation rate (A), biophysical phenomenon of the leaves as measured by *in vivo* Pulse Amplitude Modulated chlorophyll *a* fluorescence parameters deciphering the components of the thylakoid photochemistry and the photoxidative stress induced lipid peroxidation were investigated. Although, as a sign of cold stress A remarkably decreased over control, reduction was significantly more in RRII 105 than RRIM 600. Corroborating with this trend, effective PSII quantum yield (F_{PSII}) at a given light intensity, efficiency of excitation energy capture and use of photosynthetic energy in leaf photochemistry (qP) were very less in RRII 105 at low temperature than RRIM 600. In spite of down regulation of PSII activity at low temperature, there was excess flow of photosynthetic electrons across PSII and the fate of those excess electrons might decisively influence the photochemical activities right at the PSII to downstream leaf biochemistry. Depending upon the intrinsic capacity of the clones to safely handle these excess electrons at low temperature, oxidative damage occurred. Such excess photosynthetic electrons-induced photo oxidative damage at low temperature was relatively more in RRII 105 as reflected in less SOD activity and higher MDA/Chl (the ratio of MDA to chlorophyll). From overall assessment of all the above mentioned responses at physiological, biophysical and biochemical levels in low temperature stress, it appears that effective PSII quantum yield (F_{PSII}) could be a reliable tool for assessing cold stress tolerance as it can be measured in a reasonably shorter time. Therefore, judicious use of these traits for rapid probing of cold stress tolerant lines could help in crop improvement in natural rubber for cold conditions.

Key words: Rubber, cold stress, chlorophyll fluorescence, PSII activity, oxidative stress.

Introduction

It is necessary to improve the adaptation of rubber plants to low temperature, because over the past two decades, the cultivation of natural rubber has been extended to areas in cooler regions. Natural rubber has become one of the major plantation crops in the north-eastern parts of our country where prolonged low temperature in winter is a constraint for growth (Jacob *et al.*, 1999). Suboptimal temperature is a major limiting factor which results in decreased rubber yield and yield stability (Alam *et al.*, 2003; Das *et al.*, 2002; Mondal *et al.*, 1999). To overcome low temperature stress in *Hevea*, development of cold tolerant lines is very important for further crop improvement programmes and at the same time it is not an easy task for a perennial tree crop. Therefore, whether it is conventional or biotechnological approach for crop improvement, selection of intrinsic traits associated with cold stress is a most essential primary step to proceed further. In this context, exploration of such traits at physiological level can immensely help the breeders/biotechnologists to achieve the goal.

The photosynthetic apparatus of *Hevea* is known to be highly responsive to low temperature-induced photoinhibition (Alam *et al.*, 2002; Alam and Jacob, 2002; Devakumar *et al.*, 2002). Thus traits related to differential responses to low temperature- induced photoinhibitory aspects of photosynthesis could emerge as reliable tools for selecting cold tolerant lines. However, considering the economy of time and feasibility of

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handling a large populations, only those informative traits which are reliable and can be measured rapidly would be desirable. In this direction, in many annual crops e.g. maize, judicious use of chlorophyll fluorescence gained success (Andrews *et al.*, 1995; Fracheboud *et al.*, 1999). Similar approach is also feasible in rubber. Most prominent features of low temperature stress on photosynthetic apparatus is excess excitation pressure on PSII reaction centers which generally occurs whenever the absorbed light energy exceeds the capacity of the use through photosynthetic machinery or to dissipate it as heat leading to the damage of PSII and generation of reactive oxygen species resulting in oxidative stress (Huner *et al.* 1998; Fryer *et al.*, 1998).

PSII is very vulnerable to environmental stress especially in the case of low temperature concomitant with high irradiance (Adams III *et al.*, 2001; Alam and Jacob, 2002). Assessment of PSII activity through non-invasive techniques of modulated chlorophyll fluorometry can determine stress level (Fracheboud *et al.*, 1999; Schrieber *et al.*, 1998,). In this context, we present in this paper the possibility of rapid probing for informative traits associated with differential functional responses of low temperature stress in *Hevea brasiliensis*.

Materials and Methods

Two clones (RRIM 600 and RRII 105) of *Hevea brasiliensis* were used in this experiment. The plants were grown in large polybag containers (0.75 m^3) following all standard agronomic practices. To expose the plants at low temperature, one set of plants were grown on the farms of Kerala Livestock Development Board (Indo-Swiss Project) located in Mattupetty (77°4″E, 10°5″N, 1600 m msl), a hill station in the Western Ghats in the south Indian state of Kerala. The average temperature here is relatively cooler and the winter (December to March) can be very cold with occasional ground frost. A parallel set of plants was kept as control at Rubber Research Institute of India, Kottayam (76°36′E, 9°32′N).

The experiments were conducted on six months old plants during the months of January-February 2001. The average minimum temperatures for January and February 2001 in Mattupetty were 11.1 °C and 12.6 °C and the maximum temperatures were 25.4 °C and 25.2 °C respectively. The corresponding minimum temperatures in the plains of Kottayam were 22.5 °C and 23.4 °C while the maximum temperatures were 32.9 °C and 33.1 °C. Temperature below 18 °C can be stressful and therefore affect the optimal growth and yield of *Hevea* plants (Zongdao and Xueqin, 1983; Alam *et al.*, 2003). Thus, *Hevea* plants grown in Mattupetty experienced cold stress while their counterparts in Kottayam were free from it.

Photosynthetic photon flux densities (PPFD) saturated CO_2 assimilation rate (A) was measured using a Portable Photosynthesis System (Li-6200, Licor, U.S.A.). Chlorophyll *a* fluorescence was measured with a Pulse Amplitude Modulated Fluorometer (PAM-2000, Walz, Germany) following the techniques of Genty *et al.* (1989) and Schreiber *et al.* (1998). The maximum potential photochemical efficiency defined as the ratio of variable to maximum fluorescence emitted by chlorophyll (Fv/Fm) was estimated after dark adaptation of leaves for 20 minutes.

Maximal fluorescence under light exposure (Fm') was obtained by imposing a 1 s saturating flash to the leaf in order to reduce all the PSII centers after attaining a steady state fluorescence (Ft). Minimal fluorescence immediately after light exposure (Fo') was determined by imposing dark while a far-red light was simultaneously switched on to oxidize PSII rapidly by drawing electrons from PSII to PSI. Effective PSII quantum yield (F_{PSII}), efficiency of excitation energy capture by open PSII reaction center (Fv'/Fm'), photochemical (qP) and non-photochemical (qN) quenching parameters were calculated following the techniques of Genty *et al.* (1989) and Schreiber *et al.* (1998). The excess photosynthetic electrons across PSII i.e. the electrons diverted to processes not linked to photosynthetic C metabolism were also calculated (Alam and Jacob, 2002; Jacob and Karaba, 2000). Measurements were made in six to ten replications in each clone. After these measurements, the contents of chlorophyll (Chl), malondialdehyde (MDA), sugar and starch in leaves were estimated according to the methods of Arnon (1949), Heath and Parker (1968), Scott and Melvin (1953) and McCready *et al.* (1950), respectively. Antioxidant super oxide dismutase (SOD) activity was measured according to the method of Giannopolitis and Ries (1977). Standard errors were worked out and independent t test was used to find the statistical significance of the means.

Results and Discussion

There was remarkable decrease in light saturated rate of CO_2 assimilation (A) at low temperature. However, decline in A was comparatively less in RRIM 600 than RRII 105 (Fig. 1a). Corroborating with this trend in photosynthetic capacity, there was clear down regulation in the PSII activity at low temperature (Fig. 1b). There was about 23% reduction in F_{PSII} at low temperature over the control plants in RRIM 600, whereas, such reduction in F_{PSII} was as high as about

62% in RRII 105 (Fig.1b). As PSII is very sensitive to environmental stress, it has been clearly reflected in the case of *Hevea* at low temperature stress (Jacob *et al.*, 1999; Alam and Jacob, 2002). Although, the maximum potential quantum yield of PSII as measured by the dark adapted Fv/Fm was quite similar in both the clones at low temperature, it is important to note that RRIM 600 has sustained relatively higher effective PSII quantum yield (F_{PSII}) than RRII 105. Down regulation of effective PSII quantum yield in a given PPFD is an indication of photoinhibition of PSII (Adams III *et al.*, 2001).

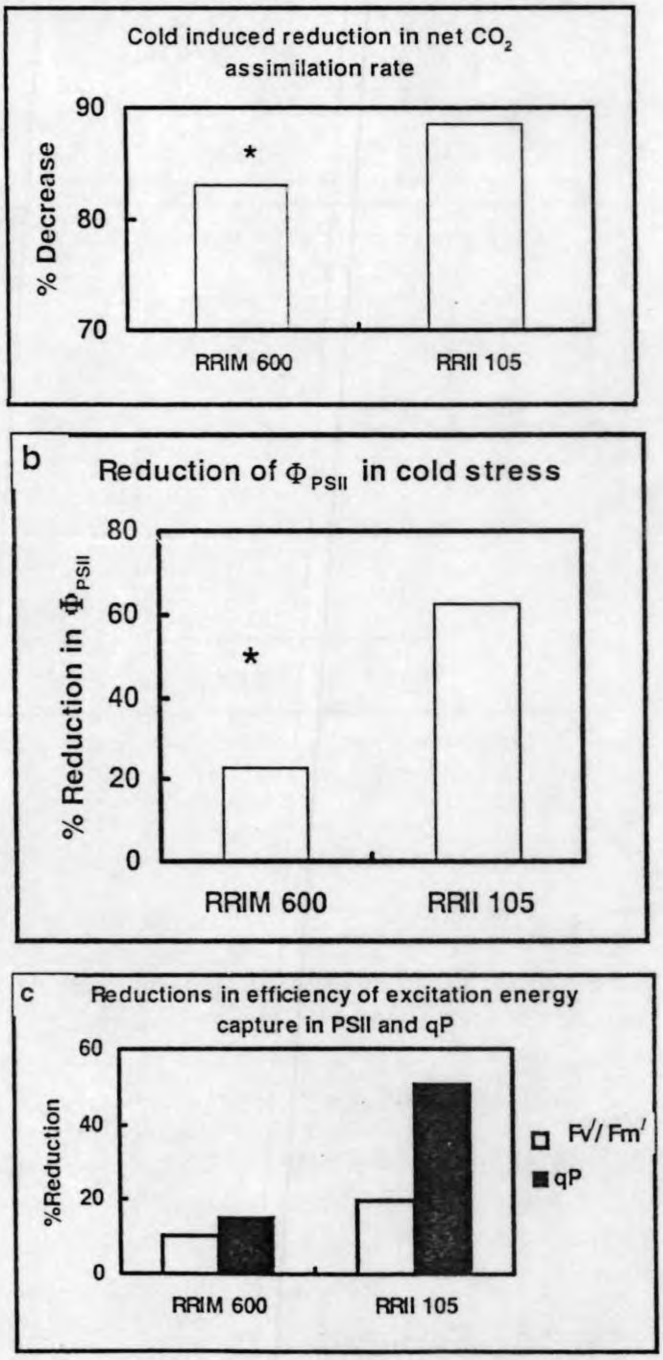

Fig. 1. Low temperature stress-induced reductions in net CO_2 assimilation rate (a), F_{PSII} (b), Fv'/Fm' and qP (c) in two clones of *Hevea*. * =significant at $p<0.05$.

The efficiency of excitation energy capture and utilisation in photochemistry largely determines the photochemical efficiency of the plants (Huner *et al.*, 1998; Allen and Ort, 2001). Low temperature stress has clearly impaired the photochemical efficiency in the present case as reflected in decline in the components of thylakoid electron transport namely efficiency of excitation energy capture by open PSII reaction center (Fv'/Fm') and photochemical quenching (qP) (Fig.1c). There was considerable excitation pressure in PSII reaction center at low temperature stress in *Hevea* as it is indicated by the increase in closed fraction of PSII reaction center (Fig.2a) and excess photosynthetic electrons (Fig.2b).

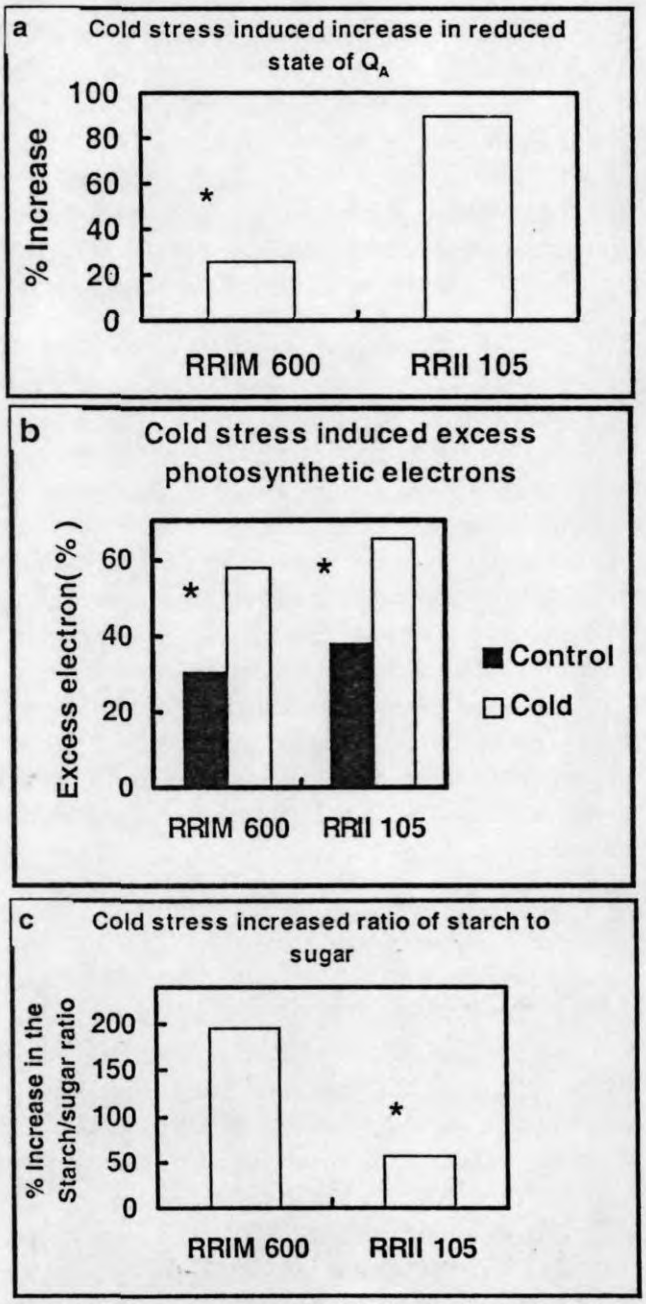

Fig. .2. Low temperature stress enhanced reduced-state of primary electron acceptor [Q_A (a)], excess photosynthetic electrons (b) and ratio of starch to sugar (c) in two clones of *Hevea*. * =significant at $p<0.05$.

Consequences of low temperature stress has also been reflected in the starch accumulation level as reflected in the ratio of starch to sugar over control plants (Fig.2c). In all these parameters, RRIM 600 exhibited relatively better performance than RRII 105 at low temperature stress.

Critical assessment of the low temperature stress responses revealed that *Hevea* plants suffered from cold and high irradiance-induced photoinhibition. The extent of photoinhibition depends on the ability of the photosynthetic apparatus either to utilise absorbed radiant energy in photochemical reactions or dissipate it safely in the light-harvesting complexes. These processes serve as sinks for excitation energy and reduce the vulnerability of PSII to photoinhibition (Melis, 1999; Allen and Ort, 2001). Therefore, an assessment of the allocation of absorbed photon energy between different processes is of considerable importance to the investigation of the strategies employed by plants to protect PSII from photoinhibition during exposure to environmental stress.

The reduction state of Q_A and the extent of thermal dissipation determined the degree of PSII photoinactivation in many environmental stresses including low temperature stress (Maxwell *et al.*, 1995; Huner *et al.*, 1998). In the case of *Hevea* also, at low temperature stress high excitation pressure resulted in the increased reduced (closed) state of Q_A and a large number of excess photosynthetic electrons. These excess electrons might induce production of AOS (active oxygen species), which resulted in oxidative stress leading to peroxidative damge as indicated in MDA/Chl ratio (Fig.3a). On the other hand, it was noted that the scavenging of these AOS was far better in RRIM 600 through enhanced SOD activity at low temperature than RRII 105 (Fig.3b).

Relatively better performance of RRIM 600 than RRII 105 in the cold prone areas in North-East India strongly supports our findings on the cold tolerant nature of the former (Meti *et al.*, 2003; Mondal *et al.*, 1999). It would appear that the clone RRIM 600 which sustained higher effective PSII quantum yield (F_{PSII}) at low temperature exhibited relatively better performance at physiological and biochemical levels for combating with cold stress-induced photooxidative stress. Thus, obviously this demonstrated that this trait is closely associated with maintenance of healthy photosynthetic apparatus, which is an essential requirement for environmental stress tolerance. From this study, it is concluded that judicious and tangible assessment of PSII activity could greatly help in probing cold tolerant lines of *Hevea* populations in crop improvement strategies for challenging environments.

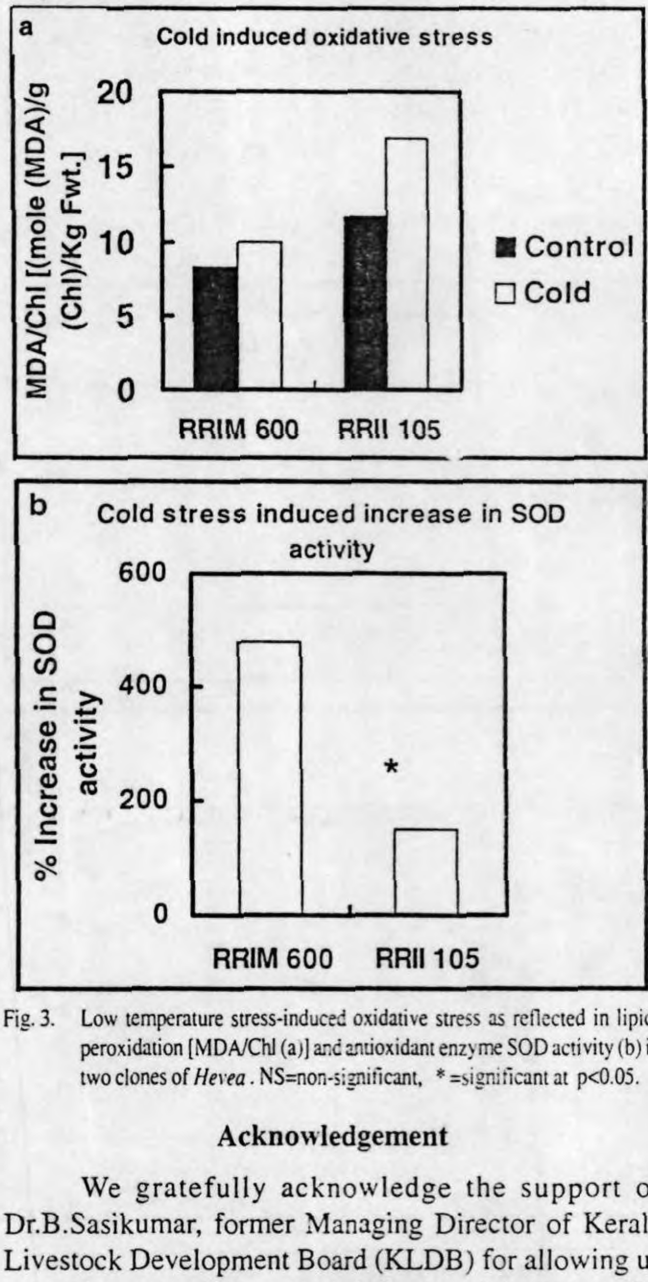

Fig. 3. Low temperature stress-induced oxidative stress as reflected in lipid-peroxidation [MDA/Chl (a)] and antioxidant enzyme SOD activity (b) in two clones of *Hevea*. NS=non-significant, * =significant at $p<0.05$.

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